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A BIO-BASED RHEOLOGY MODIFYING AGENT INSPIRED FROM NATURE

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ABSTRACT

Interaction of microorganisms and building materials, particularly with concrete and stone, were a main topic of interest for many researchers. Initially, studies focused on degradation of concrete by organic acids, produced by microbial acidification such as microbial induced corrosion. This was followed by prevention of microorganisms fouling on building materials. However, the interaction of microorganisms with materials is not necessarily negative. Recent research in the field shows that microorganisms can have positive effects on concrete and stone, such as in biological cleaning and biocalcification, which resulted with stronger and more durable materials. Further, studies revealed that it was possible to develop smart-cement based materials that could self-heal microorganisms by leveraging metabolic activity of microorganisms. Through the development of this so-called smart bio-based mortar, it became possible to improve the fresh state performance of the mix. This study focusses on design of a cement-based mortar with improved rheological properties with use of *Bacillus megaterium (B. megaterium)* and *Bacillus subtilis (B. subtilis)* cells. The bacterial cells were directly incorporated to the mix water and influence of cells on viscosity and yield strength was evaluated by rheological tests. In addition, the influence of bacteria dosage, water to cement ratio (w/c), use of superplasticizers and fly ash on performance of biological VMA were investigated. Our results showed that the apparent viscosity and yield stress of the cement-paste mix were increased with the addition of the microorganisms. Moreover, *B. megaterium* cells were found to be compatible with both fly ash and superplasticizers however *B. subtilis* were only be able to increase the viscosity when they were incorporated with superplasticizers.

Keywords: Rheology, Microorganisms, Viscosity Modifying Agent (VMA), Superplasticizers, Fly ash

1. INTRODUCTION

Recently, the use of high-performance concrete (HPC) mixes with improved workability, strength, and durability, became more popular in the field. Generally, HPC requires the use of admixtures such as superplasticizers and viscosity modifying agents (VMAs) to provide high strength without sacrificing from workability. While additives have a significant impact on most of the concrete's properties, the majority of them are chemicals and their production processes have a consequential influence on the environment. Ozcelik et al.[7] showed that while producing a concrete mixture containing 350 kg/m^3 of cement and 8.8 kg/m^3 of superplasticizer, approximately 95% of the CO_2 emissions come from cement production and 1.7% from superplasticizer production. In addition, 95% of fuel consumption was used for cement production, while 12% consumed during production of superplasticizers. Considering the impact of concrete production on CO_2 emissions and fuel consumption, chemical additives could be listed right after cement production.

Another chemical additive used in high flowable mixes is VMAs. VMAs are usually produced from polyvinyl alcohol and synthetic polymers; used to improve robustness and stability in fresh concrete [11]. Compared to superplasticizers VMAs have a similar effect on CO2 emissions and fuel consumption because they pass through the same production process. Due to these facts and concerns sustainability, increased the demand for bio-based admixtures. Research showed that usually more money spent on chemical additives in comparison with biological admixtures [14].

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Bio-based admixtures used in concrete production to improve workability, are generally produced from acrylic polymers and polysaccharide-based biopolymers [10, 12] These materials used as VMAs are mostly macromolecules containing monosaccharide side chains and anionic carboxylate groups linked to long polysaccharide chains[17]. Polysaccharide and cellulose-containing biological additives and bacterial fermentation products frequently used are welan gum [9, 14, 18], diutan gum [17] xanthan gum[14], which can increase the viscosity of fresh cement-paste by three different mechanisms [10]:

Long polysaccharide chains may absorb water and the swollen polysaccharides can increase the viscosity of the mixture water, and thus the viscosity of the cement paste. Long-chain polysaccharides can block the motion of water molecules by attracting neighbor polysaccharides near them and can form a gel that increases the viscosity of the material. When used in high doses, they may agglomerate and increase viscosity. According to these mechanisms, VMAs can reduce segregation and bleeding of fresh concrete, while providing the necessary flowability in pumping processes when used with superplasticizers [17]. Sonobi [18] studied the effect of diutan gum on the rheology of cement-based materials containing fly ash compared to welan gum and found that both additive materials could increase the viscosity and yield stress. As a result of this study, it was determined that diutan gum exhibited a higher viscosity at a low shear rate than mixtures containing welan gum, which was attributed to the molecular structure of diutan gum. In addition, when fly ash was used, a lower decrease in yield stress was observed compared to mixtures containing only diutan gum without fly ash [18].

However, both welan gum and diutan gum requires labor-intensive fermentation process that can increase the production rate and cost, which limits the use of these materials in the field [8, 14]. Another possible bio-based VMA could be bacterial cell walls. Pei et al. [13] showed that use of *Bacillus subtilis* cells walls could increase the viscosity of cement paste. However, extraction of cell walls again requires processing. In fact, bacteria are known to be VMAs not only their complicated cell wall structure, they can influence rheology as being microswimmers [16].

This paper summarizes an extensive study undertaken to investigate the possible use of *B. megateirum* and *B. subtilis* cells to improve rheology of cement-paste. Results showed that the use of cells without any physical intervention could actually increase viscosity compared to control cement paste without microorganisms. Moreover, up to a certain dosage, these microorganisms could also decrease yield strength while increasing viscosity.

2. MATERIALS and METHODS

2.1. Microorganism Selection and Growth

B. megaterium (American Typical Cell Cultures- ATCC 14581) and *B. subtilis* (ATCC 6051) were selected to be used as a VMA in cement paste. The cells were grown in a medium containing Nutrient Broth (8g) per 1liter DI water and pH was adjusted to 8. First, both *B. megaterium* and *B. subtilis* cells were inoculated in 600 mL of abovementioned nutrient medium and incubated aerobically with shaking conditions (180 rpm) at 30°C. The cells were kept under incubation until they reached to stationary phase to a cell concentration of 2×10^9 CFU/mL (~40 hours). Then, both cells were collected from the culture by centrifuging at 6300g for 15 min. Then the cells were washed twice by PBS (Phosphate buffered solution) and kept at 4°C until testing.

2.2. Preparation of Cement Paste Samples

Cement paste samples were prepared by Ordinary Portland Cement (OPC) CEM I 42.5 R. To evaluate the influence of w/c ratio on the efficiency of cells, the mixtures were prepared with 2 different w/c ratios: 0.36 and 0.50. In addition, to determine the effects of superplasticizers and fly ash on the performance of the biological VMA, a polycarboxylate superplasticizer (by 0.1 kg/kg cement) and Ftype fly ash (by 20% of the cement weight) was added to samples. To investigate the effect of microorganisms on the rheology of the cement paste, the abovementioned collected *B.megaterium and B. subtilis* (*see* Section 2.1) cells were directly added to the mixing water. The number of cells were adjusted in terms of percent weight of cement, such as 0.05% and 0.10%, of the cement weight. The cement paste samples were prepared according ASTM C305-14 Standard Practice for Mechanical Mixing of Hydraulic Cement Pastes and Mortars [3]. The cells were added to mixing water prior to mixing, homogenized with hand-mixing for 30s, and then mixed with cement. In case of superplasticizer addition, the cells were added to initial 2/3rd of the mix water and the superplasticizer was added to the 1/3rd of the mix water. The last 1/3rd protion of the mix water including superplasdticizer was added during the last 60s of the mixing.

2.2. Rheological Measurements

Upon mixing, the cement paste samples were first mixed for 60 seconds to ensure the homogeneity of the mixtures. Then, a pre-shear stage where the shear rate was kept constant at 100s^{-1} for another 60s. Following the pre-shear, the analysis was conducted by increasing the shear rate from 100s^{-1} to 1s^{-1} and the yield stresses and viscosity were recorded. The upcurve was chosen for evaluation of the rheological behavior of the samples. The rheological behavior of the cement paste was evaluated using the Bingham model ($\tau = \tau_o + \mu\dot{\gamma}$). Where τ_0 is the yield stress (Pa), μ is the plastic viscosity (Pa.s), and $\dot{\gamma}$ is the shear rate (s^{-1}).

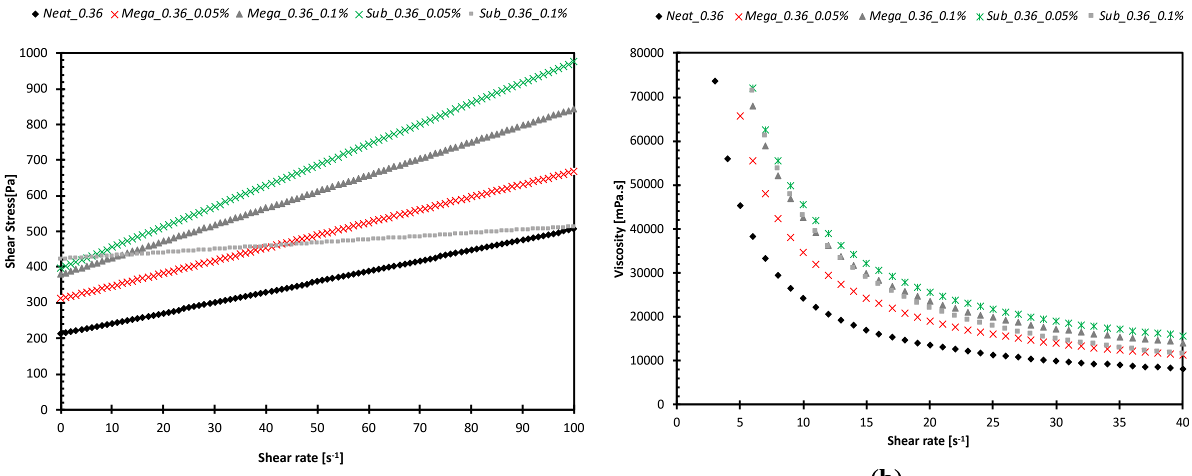
An alternative model also should be considered, since the shear-thinning or shear- thickening behavior of mixes were unknown. In case of a shear thickening behavior, the flow curve would not be simply explained by Bingham equation. Instead, a modified Bingham equation was used to analyze shear-thickening cement paste samples ($\tau = \tau + \mu\gamma \dot{+} c\dot{\gamma}^{n}$), which could also explain the shear thinning behavior as well [6]. Where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), μ is plastic viscosity, $\dot{\gamma}$ is the shear rate (s^{-1}) and c is second degree parameter (Pa.s^2) [6]. When c/ μ > 0 the materials were classified as shear-thickening and when c/ μ < 0, the materials exhibited shear thinning behavior.

To evaluate the time-dependent behavior of cement-paste, the samples were re-tested by increasing the shear rate from 100s^{-1} to 1s^{-1} without premixing after 10 minutes of waiting and the change in viscosity was recorded. This waiting period simply corresponding to 20 minutes upon mixing which is mainly initiation of induction period [4].

3. RESULTS and DISCUSSION

This study was undertaken to evaluate the influence of *B. megaterium* and *B. subtilis* cells on rheological properties of cement paste. Figure 1 to Figure 3 show the yield stress and viscosity curves under increasing shear stress for cement paste samples including different bacteria content, fly ash (20% by weight of cement) and superplasticizer (1% of cement weight) at a w/ c of 0.36.

All cement paste samples exhibited a shear-thinning behavior regardless of the bacteria dosage and could be modelled by Bingham equation (*see* Section 2.2). Incorporation of microorganisms did not change the material behavior such that the cement paste samples containing bacterial cells also exhibited a shear-thinning response. Addition of cells increased the viscosity of the cement paste regardless of the w/c. This might be directly related to increasing molecular weight of polysaccharides and peptidoglycans in the cells structure resulting in an increasing intertwining of chains and leading to a higher water retention at lower shear-rates [12]. However, with increasing shear rate the chains would break and releasing the water to the mix, leading to a more pronounced decrease in viscosity compared to low shear rates. In addition, these cells are negatively charged, thus they may also interact with calcium ions present in the environment and absorbed on the cement particles. This might improve the flocculation and agglomeration of the solids in the mix, reducing the flowability. However, with increasing shear rate the chains (or bonds) would break and releasing the water to the mix, leading to a more pronounced decrease in viscosity compared to low shear rates. In particular, the *B. subtilis* cells were found to be more efficient in terms of increasing the viscosity. Previously, Pei et al.[13] observed an increase in apparent viscosity approximately 40% when only *B. subtilis* cell walls (0.34% by weight of cement) were incorporated in cement paste at a w/c of 0.50 at low shear rates (<40 s^{-1}). Herein, the use of vegetative *B. megaterium* (at a dosage of 0.1% by cement weight) and *B. subtilis* (at a dosage of 0.1% by cement weight) cells resulted with a 62% increase at corresponding shear rates. The difference of using active cells compared to the incorporation of only bacterial cells walls could be explained by the theory of pushing and pulling effect of bacteria cells. Rafaï et al. [15] showed that motile microalgae cells could increase the effective viscosity of a suspension. Moreover, the live cells showed a significantly higher efficiency than the dead cells, which suggested that the behavior was related to motility rather than cell structure [15]. Herein, the vegetative cells also showed a higher efficiency compared to what has been found in the literature, thus this might indicate that motility of these cells could actually impose a puller effect, also inducing additional resistance to flow. Moreover, the resistance of cells to higher shear rates. Further research has to be conducted to analyze the kinematics of vegetative gram-positive bacterial cells and how they actually influence the hydrodynamics of cement paste suspension.

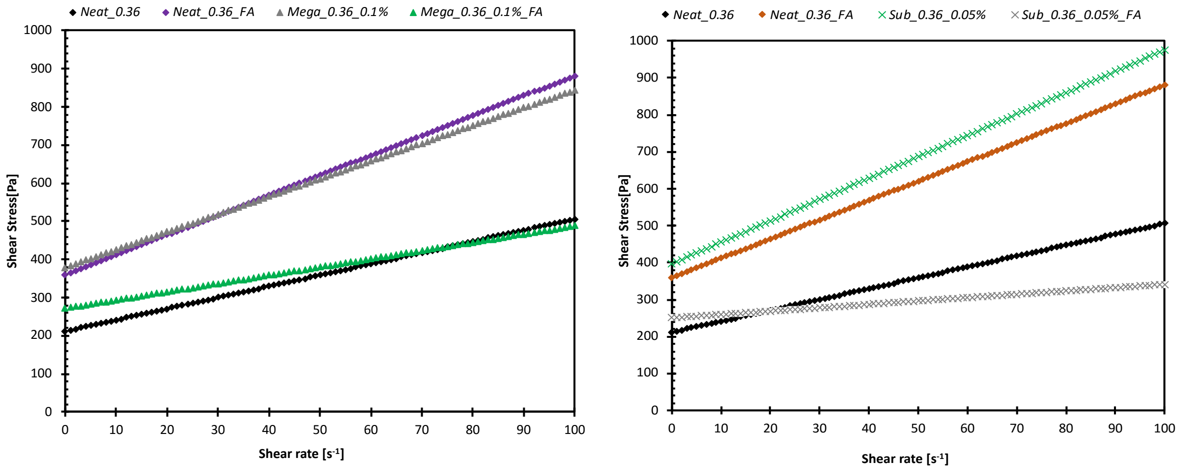


(a) **(b)**

Figure 1. Rheological test results for cement paste samples at a w/c of 0.36 (a)shear stress response with increasing shear rate (b) change in viscosity with increasing shear rate. Cell dosages were used as 0.05% and 0.1% per weight of cement. *Mega: Bacillus megaterium* and *Sub: Bacillus subtilis.*

Comparing the efficiencies of these 2 different strains, *B. megaterium* showed a better performance compared to *B. subtilis* considering their compatibility with fly ash and superplasticizers (*see* Figure 2 and 3). *B. megaterium* is a gram-positive strain having a thick cell wall and long rod-shaped with chain-like arrangement bacteria [2]. In particular, the “megaterium” name was given because there are one of the largest bacteria in the soil and it is classified in *Bacillus sp*. due to its ability for forming endospores and resistance to extreme conditions [19]. Yet, this strain was selected due to its size and thick cell wall, which presumably have a relatively high volume of polysaccharides and peptidoglycans. *B. megaterium* cells were used to improve strength of mortar [1, 2] but their influence on rheology is not known. As so, *B. megaterium* cells were able to increase the viscosity regardless of the w/c and bacteria dosage used in the mix.

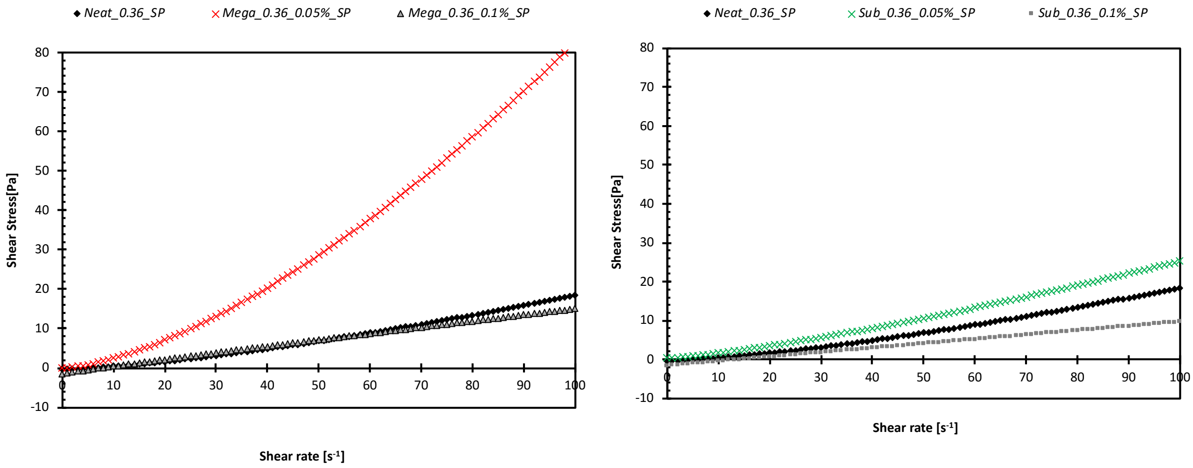
The interaction of cells with fly ash exhibited a rather different trend, especially at low w/c. Since fly ash is finer than cement particles, adding fly ash to the mixture reduces the inter-particle distances and increases the pressure between particles. While this pressure leads to the removal of the water from inter-particle spaces, it also provides a flowing effect on cement paste [5]. Thus, incorporation of fly ash generally improves workability and provides stability with increasing viscosity, and in some cases the yield strength of the paste. Incorporation of fly ash by 20% for the weight of cement only influenced the viscosity and the yield stress of cement paste at a w/c of 0.36 such that both of these parameters were higher compared to its counter par near cement paste. In such a case, the fine fly ash particles acted as a filler material since there was not enough fluidity that would enable the spherical particles to flow and improve workability. However, at higher w/c, the interaction of cells with fly ash was similar to the samples without any fly ash. Yet, the exact reason is not known, the interaction of fly ash and cells might depend on (a) particle packing density (b) workability of the mix and (c) molecular weight of the cells.



(a) **(b)**

Figure 2. Rheological test results for cement paste samples including 20% fly ash (FA) by cement weight at a w/c of 0.36 (a) including *Bacillus megaterium* cells (b) including *Bacillus subtilis* cells. Cell dosages were used as 0.05% and 0.1% per weight of cement.

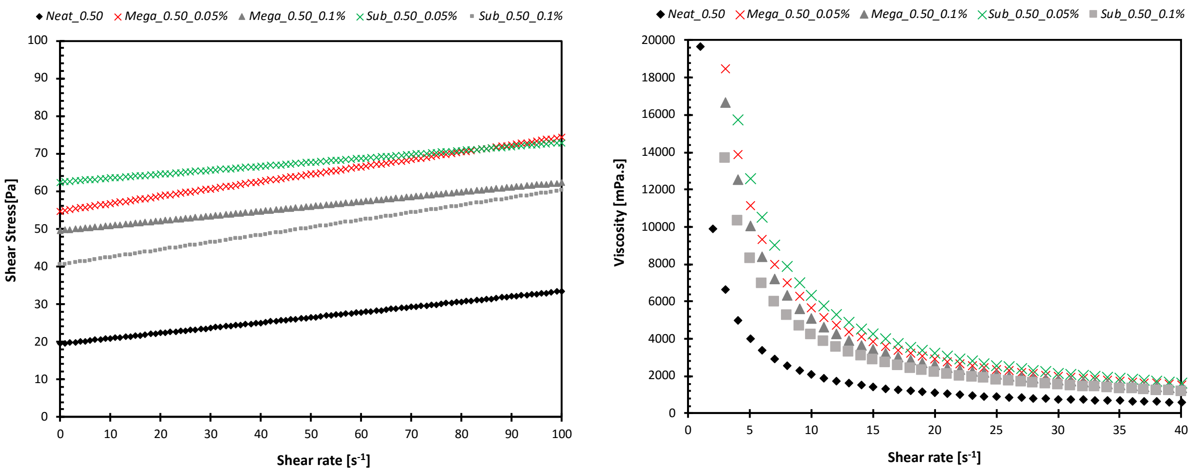
The higher efficiency of *B. megaterium* compared to *B. subtilis* when they were incorporated with superplasticizers could be explained by its cell size and composition. Yet, this strain was selected due to its size and thick cell wall, which presumably have a relatively high volume of polysaccharides and peptidoglycans. Thus, it might lead to higher water retention leading to a higher increase in yield stress and viscosity**.**



(a) **(b)**

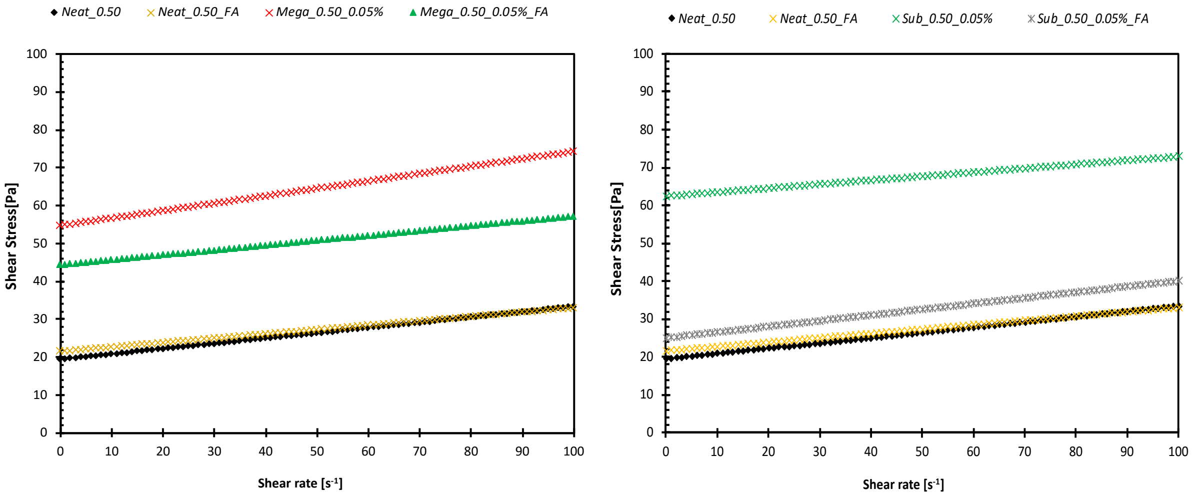
Figure 3. Rheological test results for cement paste samples including 1% superplasticizers (SP) by cement weight at a w/c of 0.36 (a) including *Bacillus megaterium* cells (b) including *Bacillus subtilis* cells. Cell dosages were used as 0.05% and 0.1% per weight of cement.

The influence of microorganisms as a VMA was less pronounced at 0.50 (see Figure 4). Herein, both *B. megaterium* and *B subtilis* cells showed a similar behavior. This was directly related to increasing the fluidity of the mix. Even though the flowability of the mix increases, the bacterial cells were able to increase the viscosity of the material by 7% event with of 0.05% cells by weight of cement. It is simply the cells could not overcome the high fluidity of the mix. The increased amount of water content in the mix that might limit the attractive forces between the strains chain [14]. Even in this case, the viscosity of the mix was even the doubled when bacteria dosage was increased to 0.1% of cement weight. Similar to low w/c samples, *B. megaterium* exhibited a higher efficiency compared to *B. subtilis* in FA amended samples at a w/c of 0.50 (see Figure 5). In fact, in both cases increase in *B. subtilis* concentration resulted with lower viscosities even compared to neat paste. Further rheological studies should be conducted with different instrument geometries and measuring methods to actually define the exact influence of cells on rheology and their interaction with FA.



(a) **(b)**

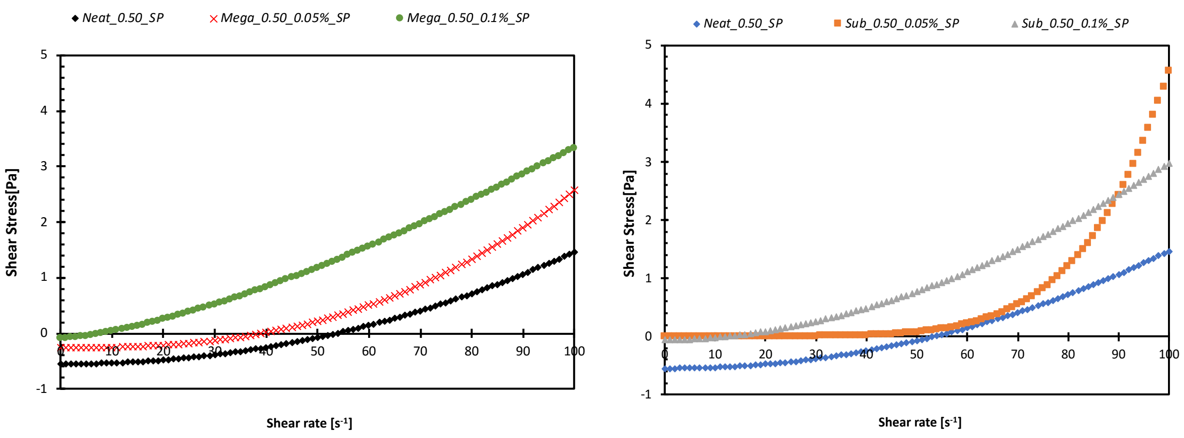
Figure 4. Rheological test results for cement paste samples at a w/c of 0.50 (a)shear stress response with increasing shear rate (b) change in viscosity with increasing shear rate. Cell dosages were used as 0.05% and 0.1% per weight of cement. *Mega: Bacillus megaterium* and *Sub: Bacillus subtilis.*



(a) **(b)**

Figure 5. Rheological test results for cement paste samples including 20% fly ash (FA) by cement weight at a w/c of 0.50 (a) including *Bacillus megaterium* cells (b) including *Bacillus subtilis* cells. Cell dosages were used as 0.05% and 0.1% per weight of cement.

Incorporation of superplasticizers completely changed the rheological behavior of cement paste samples regardless of the w/c used (see Figure 6). The use of a very strong polycarboxylate superplasticizer resulted with a shear-thickening behavior rather than shear thinning behavior previously observed in rest of the samples. In this case, the shear-thickening cement paste behavior mix would not be simply explained by Bingham equation (see Section 2.3). Instead, a modified Bingham equation was used to analyze shear-thickening cement paste samples [6]. In addition, high flowability in these samples, particularly in neat control paste, lead excessive segregation which might interfere test results. This effect was also eliminated at low shears along with addition of cells. It should be noted that, the dosage of superplasticizer was kept constant (0.1% by cement weight) even at high w/c mixes (0.50), which yielded a very flowable mix. Moreover, incorporation of a very strong superplasticizer yielded negative viscosity values at very slow shear rates. High flowability of the mix resulted with inconsistencies in rheological parameters such that negative viscosity values were recorded at low shear rates. This might indicate possible segregation in the mix and leading to invalid test results. Thus, any increase in viscosity at low shear rates might also be an indication of reduced segregation. While use of superplasticizer the neat paste with a w/c of 0.50 lead to an instable rheological in the mix (*see* Figure 6), incorporation of cells rather increased the viscosity and reduced the instability, leading to a more homogenous behavior.



(a) **(b)**

Figure 6. Rheological test results for cement paste samples including 1% superplasticizers (SP) by cement weight at a w/c of 0.50 (a) including *Bacillus megaterium* cells (b) including *Bacillus subtilis* cells. Cell dosages were used as 0.05% and 0.1% per weight of cement.

4. CONCLUSIONS

This study represented the results of an extensive study undertaken to evaluate the possible use of microorganism VMA, or more precisely as a rheology modifying agent. The flow behavior of cement paste samples including *B. megaterium* and *B. subtilis* cells were evaluated through rheological tests. Moreover, the compatibility of cells with the use of superplasticizer and fly ash, as well as the influence of w/c were investigated. Incorporation of cells increased the plastic viscosity regardless of the w/c used for the mixes. This influence was attributed to the interwinding of peptidoglycan chains leading to an increase in viscosity of mix water. Increasing w/c lead to a decrease in the performance of cells in terms of being a RMA. The influence of cells was much more pronounced at a w/c of 0.36 than it was at 0.50. *B. megaterium* showed a better performance compared to *B. subtilis* considering their compatibility with fly ash and superplasticizers. While *B. megaterium* cells were compatible with both additives *B. subtilis* cells were not be able to improve viscosity in mixtures prepared by superplasticizers, particularly at high w/c. This novel study could be considered as preliminary evaluation of a bio-based grout with improved rheology. Although, it might not be possible to drive a quantitative statement in terms of increase in viscosity, we could still conclude that the bacterial cells, particularly *B. megaterium*, could serve as

VMAs in cement-based materials. Further studies have to be conducted to understand the other possible parameters of cells influencing the rheology such as the effect of cells on drag forces and particle-to-particle interaction. With explotion of these mechanisms will lead to possible use of this grout in practicle applications.

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REFERENCES

[1] Achal V, Pan X, Özyurt N Improved strength and durability of fly ash-amended concrete by microbial calcite precipitation. Ecol Eng, 2011; 37:554–559. doi: 10.1016/j.ecoleng.2010.11.009

[2] Andalib R, Abd Majid MZ, Hussin MW, Ponraj M, Keyvanfar A, Mirza J, Lee HS Optimum concentration of Bacillus megaterium for strengthening structural concrete. Constr Build Mater 2016; 118:180–193. doi: 10.1016/j.conbuildmat.2016.04.142

[3] ASTM C305 Standard Practice for Mechanical Mixing of Hydraulic Cement Pastes and Mortars of Plastic Consistency. ASTM lnternational, 2011; 1–3. doi: 10.1520/C0305-13.2

[4] Basaran Bundur Z, Kirisits MJ, Ferron RD. Biomineralized cement-based materials: Impact of inoculating vegetative bacterial cells on hydration and strength. Cem Concr Res, 2015; 67:237–245. doi: 10.1016/j.cemconres.2014.10.002

[5] Esmaeilkhanian B, Feys D, Khayat KH, Yahia A. New test method to evaluate dynamic stability of self-consolidating concrete. ACI Mater J, 2014; 111:299–307. doi: 10.14359/51686573

[6] Feys D, Verhoeven R, De Schutter G Fresh self compacting concrete, a shear thickening material. Cem Concr Res, 2008; 38:920–929. doi: 10.1016/j.cemconres.2008.02.008

[7] G Ozcelik, AP Gürsel ÇM Yap ı Kimyasallar ı na Çevresel Çerçeveden Bak ış. In: Uluslararası Katılımlı Yapılarda Kimyasal Katkılar 4. Sempozyumu ve Sergisi, 2013; pp 179–197

[8] Ivanov V, Chu J, Stabnikov V. Basics of Construction Microbial Biotechnology. In: Pacheco-Torgal F, Labrincha JA, Diamanti MV, Yu C (eds) Biotechnologies and Biomimetics for Civil Engineering. Springer, 2015; pp 21–56

[9] Jiménez M, Mateo R, Mateo JJ, Huerta T, Hernández E. Effect of the incubation conditions on the production of patulin by Penicillium griseofulvum isolated from wheat. Mycopathologia, 1991;115:163–168. doi: 10.1007/BF00462220

[10] Khayat KH Viscosity-enhancing admixtures for cement-based materials — An overview. Cem Concr Compos, 1998; 20:171–188. doi: 10.1016/S0958-9465(98)80006-1

[11] Kurdowski W. Cement and Concrete Chemistry, 2014; Springer, Dordrecht. https://doi.org/10.1007/978-94-007-7945-7

[12] Lachemi M, Hossain KMA, Lambros V, Nkinamubanzi PC, Bouzoubaâ N. Self-consolidating concrete incorporating new viscosity modifying admixtures. Cem Concr Res, 2004; 34:917–926. doi: 10.1016/j.cemconres.2003.10.024

[13] Pei R, Liu J, Wang S. Use of bacterial cell walls as a viscosity-modifying admixture of concrete. Cem Concr Compos, 2015; 55:186–195. doi: 10.1016/j.cemconcomp.2014.08.007

[14] Plank J. Applications of biopolymers and other biotechnological products in building materials. Appl Microbiol Biotechnol, 2004; 66:1–9. doi: 10.1007/s00253-004-1714-3

[15] Rafaï S, Jibuti L, Peyla P. Effective Viscosity of Microswimmer Suspensions. Phys Rev Lett, 2010; 104:1–4. doi: 10.1103/PhysRevLett.104.098102

[16] Saintillan D Kinetic models for biologically active suspensions. 2012; Urbana, ill.

[17] Schmidt W, Brouwers HJH, Kühne HC, Meng B The working mechanism of starch and diutan gum in cementitious and limestone dispersions in presence of polycarboxylate ether superplasticizers. Appl Rheol, 2013; 23:1–12. doi: 10.3933/ApplRheol-23-52903

[18] Sonebi M Rheological properties of grouts with viscosity modifying agents as diutan gum and welan gum incorporating pulverised fly ash. Cem Concr Res, 2006; 36:1609–1618. doi: 10.1016/j.cemconres.2006.05.016

[19] Vary PS, Biedendieck R, Fuerch T, Meinhardt F, Rohde M, Deckwer WD, Jahn D Bacillus megaterium-from simple soil bacterium to industrial protein production host. Appl Microbiol Biotechnol, 2007; 76:957–967. doi: 10.1007/s00253-007-1089-3